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This Position Document addresses the risks and benefits of pesticide products containing the subject active ingredient. The Agency has determined that the use of products containing the subject active ingredient may meet or exceed a risk criterion described in 40 CFR Part 154. Potential hazards will be examined further to determine the nature and extent of the risk, and considering the benefits of the subject active ingredient, whether such risks cause unreasonable adverse effects on the environment.

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Decision Document
on
Dichlorvos

Office of Pesticides and Toxic Substances
United States Environmental Protection Agency
Washington, D.C.
September 30, 1982

Executive Summary

Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (FIFRA) (7 U.S.C. Section 136 et seq.), the Environmental Protection Agency (EPA or the Agency) regulates all pesticide products. Section 6(b) of FIFRA authorizes the Administrator of the EPA to issue a notice of intent to cancel the registration of a pesticide or to change its classification if it appears to him that the pesticide or its labeling "does not comply with the provisions of [FIFRA] or, when used in accordance with widespread and commonly recognized practice, generally causes unreasonable adverse effects on the environment".Section 3(c)(6) authorizes the Administrator to deny applications for pesticide registration if the statutory standards for registration are not met.

The Agency designed the Rebuttable Presumption Against Registration (RPAR) process to gather risk and benefit information about problem pesticides and to make balanced decisions concerning them in a manner which allows all interested groups to participate. This process is set forth in 40 CFR 162.11.

Dichlorvos was originally referred to the Special Pesticide Review Division because scientific studies indicated that dichlorvos was mutagenic and might cause cancer, nerve damage and birth defects in laboratory animals.

All registrants were notified by registered mail in September 1980, that the Agency had determined that all pesticide products containing dichlorvos were to be reviewed as possible candidates for the issuance of the RPAR.

Dichlorvos is an organophorous pesticide used to control a number of different species of insects affecting public health, animals and plants. It is used as an insecticide in domestic dwellings, dairy barns, tobacco warehouses, mushroom houses, and inside aircraft to control ants, cockroaches, fleas, flies, gnats, mosquitoes, and ticks. Dichlorvos is formulated as emulsifiable concentrates, soluble concentrate liquids, granulars, pressurized liquids and dusts, impregnated materials, pellets/tablets, wettable powders, and dusts.

The Agency reviewed four studies on the oncogenic potential of dichlorvos. No positive evidence of oncogenicity was identified. The studies were, however, all flawed, requiring additional information. The National Cancer Institute (NCI) is currently conducting an evaluation of the oncogenic potential of dichlorvos. No additional data will be required of the registrants until the Agency has reviewed the results of the NCI study.

The Agency reviewed the mutagenicity data on dichlorvos. There was extensive evidence that dichlorvos was mutagenic in bacteria in the absence of a mammalian activation system. There was also suggestive evidence that dichlorvos was mutagenic in fungi. However, no mutagenic effects of dichlorvos were detected in mammalian systems. Additional testing will be required to confirm the lack of mutagenic effects in mammalian systems.

An evaluation of the available data revealed no definitive evidence or valid tests suggesting that dichlorvos induced teratogenic or fetotoxic effects in the absence of maternal toxicity.

An evaluation of the reproductive effects data revealed no definitive evidence or valid tests suggesting dichlorvos had any adverse effect on fertility or other reproductive parameters.

The available information was sufficent to show that dichlorvos did not produce organophosphate-type delayed neurotoxicity.

Based on the above information, the Agency has determined that the existing evidence does not support the issuance of an RPAR for dichlorvos.

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I. INTRODUCTION

A. General

This document represents the conclusions of the Environmental Protection Agency (the Agency) that the pesticide dichlorvos does not exceed the criteria for issuance of a Rebuttable Presumption Against Registration (RPAR). The background and rationale for this decision are detailed in the following sections of the document.

This decision document is divided into four sections. This introduction (Section I) presents the legal background and the regulatory history of dichlorvos. Section II (Chemical Profile) discusses the pesticide's chemistry, uses, and tolerances. Section III discusses dichlorvos as an RPAR candidate; it examines and summarizes dichlorvos' adverse effects data in relation to the Agency's RPAR criteria and contains various conclusions. Section IV presents the Agency's conclusions regarding the issuance of the RPAR. A bibliography of the studies cited is also included.

B. Legal Background

1. General

Section 3(a) of the Federal Insecticide Fungicide and Rodenticide Act of 1978 (FIFRA) requires the registration of all pesticide products prior to sale or distribution. Section 6(b) of FIFRA authorizes the Administrator to issue a notice of intent to cancel the registration of a pesticide or to change its classification if it appears that the pesticide or its labeling "does not comply with the provisions of [FIFRA] or, when used in accordance with widespread and commonly recognized practice, generally causes unreasonable adverse effects on the environment." FIFRA Section 2 (bb) has defined "unreasonable adverse effects on the environment" to mean "any unreasonable risk to man or the environment, taking into account the economic, social and environmental costs and benefits of the use of any pesticide."

The Agency created the RPAR process to facilitate the identification of pesticide uses which may cause unreasonable adverse effects on the environment and to provide a public, informal procedure for the gathering and evaluation of information about the risks and benefits of these uses. The regulations governing the RPAR process are set forth in 40 CFR 162.11. In summary, these regulations set forth certain criteria of risk and provide that the RPAR shall be issued against a pesticide if the Agency determines that the ingredient(s), metabolite(s), or degradation product(s) of the pesticide in question meet or exceed any of these risk criteria.

In administering the RPAR process, the Agency adheres to the standard for initiating the RPAR process established by Section 3(c)(8), of the FIFRA (1978) amendments, which provides that the Agency may not start an RPAR unless it has "a validated test or other significant evidence raising prudent concerns of unreasonable adverse risk to man or the environment". In determining whether a particular pesticide raises "prudent concerns", the Agency examines the degree of toxicity of the pesticide, as well as the likelihood of human and environmental exposure. This approach allows the Agency to avoid the burdensome consequences of RPAR proceedings in those situations in which the uses of the pesticide at current levels of exposure do not appear to pose an unreasonable risk to man or the environment.

Where the Agency publishes a notice indicating that the RPAR has been issued, registrants, applicants, and interested persons must be provided an opportunity to submit rebuttal evidence including benefits information relating to any use of the pesticide. If the presumptions of risk are not rebutted, the benefits of the pesticide are evaluated and considered along with the information on the competing risks. The Agency then analyzes various methods of reducing the risk from use of the pesticide, and determines whether such methods reduce risk to a level which is outweighed by the benefits of continued use. If measures short of cancellation cannot reduce the risks of the pesticide to a level which is outweighed by the benefits of the use, the use in question must be cancelled.

2. Rebuttable Presumption/Risk Criteria (40 CFR 162.11)

40 CFR 162.11 (a)(3)(ii) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s):

..."Induces oncogenic effects in experimental mammalian species or in man as a result of oral, inhalational, or dermal exposure."

As described in the Proposed Guidelines for Registering Pesticides in the U.S., 40 CFR 163.83-1,-2 (43 FR 37346, August 22, 1978), oncogenicity testing "focuses on the detection of malignant and benign tumors and preneoplastic lesions" in two mammalian species. One species shall normally be a generally recognized strain of laboratory rat.

..."Induces mutagenic effects, as determined by multitest evidence."

40 CFR Section 162.3(y) defines mutagenicity as "the property of a substance or mixture of substances to induce changes in the genetic complement of either somatic or germinal tissue in subsequent generations." Mutagenic chemicals are recognized as posing a potential risk to human health because of their ability to cause heritable changes in genes and chromosomes. Such germline changes can, for example, lead to birth defects or to the accumulation of deleterious mutations in the human gene pool. In addition, somatic mutations may be involved in the etiology of cancer.

..."Produces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account an ample margin of safety."

Risk criteria are also defined in other sections of 40 CFR 162.11 to include hazards to wildlife [162.11(a)(3)(i)(B)], and acute toxicity [162.11(a)(3)(i)].

C. Referral Background

Dichlorvos was originally referred to the RPAR process because scientific studies indicated that dichlorvos is mutagenic and may cause cancer, nerve damage and birth defects in laboratory animals.

All registrants were notified by registered mail in September 1980, that the Agency had determined that all pesticide products containing dichlorvos were to be reviewed as possible candidates for the issuance of an RPAR (Williams, 1980).

D. Health Effects Monitoring

The Pesticide Incident Monitoring System (PIMS) was developed to collect data on the effects of pesticides on man and the environment. The incidents reported through the PIMS are synopses collected from a variety of sources and lack uniformity in detail and confirmation of the incident.

As part of its assessment of dichlorvos, the Agency reviewed its extensive PIMS data base covering a period from 1964 to December, 1980. Only 182 of the 598 dichlorvos incidents could be identified as involving products that contained dichlorvos as the sole active ingredient and, hence, are of special interest to the Agency's assessment. A majority (147) of these 182 reports involved humans and domestic animals in the home environment, with 114 incidents resulting from ingestion and application of dichlorvos. Ingestion incidents usually involved children chewing flea collars and "no pest strips". Most of the application incidents involved situations where the existing label precautions were not followed. A review of the labeling indicated that the human and domestic animal hazard labeling is currently adequate.

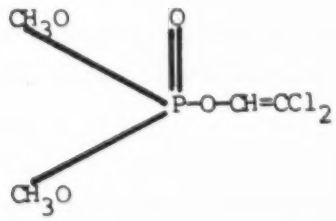
This review of the PIMS failed to locate any incidents of health effects related to the RPAR criteria.

II. CHEMICAL PROFILE

This section presents the background of dichlorvos, its chemical properties as a possible metabolite of naled and trichlorfon, and pesticide registration information.

A. Chemical Composition

The chemical name for dichlorvos is 2,2-dichlorovinyl dimethyl phosphate. The Chemical Abstract Service registry number for dichlorvos is 62-73-7. The structural formula of dichlorvos is shown below:



Dichlorvos is produced by heating chloral, trimethyl phosphite and suitable inert solvents at atmospheric pressure (Whetstone, R.; Harman, D. U.S.Patent 2,956,073, 1960).

Dichlorvos is an organophosphorous pesticide used to control a number of different species of insects affecting humans, animals and plants. It is formulated as emulsifiable concentrates, soluble concentrate liquids, granulars, pressurized liquids and dusts, impregnated materials, pellets/tablets, liquids (ready to use), wettable powders and dusts. This chemical is marketed under several trade names. Some examples are: Dedevap®, Nuvan®, Atgard®, No-pest®, Vapona®, and Vaponite®.

B. Dichlorvos as Related to Naled and Trichlorfon

Naled (1,2-dibromo-2,2-dicloroethyl dimethyl phosphate) and trichlorfon (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) are organophosphorous pesticides.

Trichlorfon and naled can both degrade to dichlorvos. Under alkaline conditions, trichlorfon chemically rearranges to form dichlorvos. In the presence of metals and reducing agents, naled will lose bromine and if hydrolyzed, will form dichlorvos. No evaluation of the likelihood of these conversions under actual use conditions has been conducted. However, the issue will be considered fully during the generic reviews of trichlorfon and naled.

C. Registered Products, Uses, and Tolerances

The Agency has currently issued 942 registrations from 255 registrants for dichlorvos containing products. (Product Label File: Dichlorvos 1982).

Dichlorvos is registered for use as a pesticide in domestic dwellings, dairy barns, tobacco warehouses, mushroom houses, aircraft and other areas where flies, mosquitoes, gnats, cockroaches, fleas, ticks, ants and crickets may be a problem.

Tolerances for residues of dichlorvos have been established on the following raw agricultural commodities (40 CFR 180.235):

Cattle, eggs, goats, horses, milk, poultry, radishes, sheep, swine and non-perishable packaged or bagged raw agricultural commodities and on nonperishable bulk stored raw agricultural commodities, regardless of fat content.

In addition there are tolerances for the use of dichlorvos on cucumbers, lettuce, mushrooms and tomatoes, which are expressed as naled.

As a food additive, 2,2-dichlorovinyl dimethyl phosphate may be present as a residue from application as an insecticide on packaged or bagged nonperishable processed food in an amount not in excess of 0.50 part per million (21 CFR 193.140).

III. DICHLORVOS AS RPAR CANDIDATE

This section examines the available data on dichlorvos. The Agency evaluates the results of these reviews in relation to the established risk criteria and concludes the review of dichlorvos as an RPAR candidate within this section.

A. Oncogenicity

1. Introduction

40 CFR Section 162.11 (a)(3)(ii)(A) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s) ..." induces oncogenic effects in experimental mammalian species or in humans as a result of oral, inhalation, or dermal exposure..." Section 162.3 (bb) defines the term oncogenic as "the property of a substance or a mixture of substances to produce or induce benign or malignant tumor formation in living animals."

2. Data Review

The potential oncogencity of dichlorvos in rodents has been examined in four chronic studies using either the oral or inhalational exposure route. In three of the studies, the results were considered negative. However, in each case, design flaws and poor survival rates combined to produce results considered inadequate to definitively support the negative oncogenic potential of dichlorvos. In the fourth study, a National Cancer Institute (NCI) bioassay, the results were considered equivocal and dichlorvos is currently being retested by NCI.

a. Blair et al., 1976

The first study reviewed was an inhalation carcinogenesis study in rats reported by Blair et al. (1976). Groups of 100 Carworth Farm E (CFE) strain of rats (50 of each sex) were exposed to 0.05, 0.5, or 5.0 mg/m^3 of technical dichlorvos (greater than 97 percent purity) in 10-m^3 chambers. The rats were five weeks old at the start of the study, and the study was designed so that 10 males and 10 females (randomly chosen) were placed in the chambers each week over a five week period.

The dichlorvos chamber atmosphere was generated using the method of Blair and Rees (1975). Chamber concentrations were determined daily using gas chromatography with a phosphorus specific thermionic detector. Average chamber dichlorvos concentrations over the test period were 4.70, 0.48, and 0.05 mg/m^3, and compare favorably with the nominal concentrations of 5.0, 0.5, and 0.05 mg/m^3 respectively. Animals were exposed 23 hours a day each day for 100 weeks (males) or 104 weeks (females).

Necropsies were performed on all rats in the study, and tissues were taken for microscopic examination. These tissues consisted of "major viscera," macroscopic tumors, and blocks of tongue, nasal cavity, trachea, skeletal muscle, eye, and lachrymal gland. Tumor data were examined by actuarial analysis (Cox, 1972; Mantel, 1966; Peto and Peto, 1972; and Peto, 1974).

The growth rate of all treated rats was depressed to some extent, but was more prominent in males. At terminal sacrifice, plasma and brain cholinesterase activity was significantly depressed in rats of both sexes from the 0.5 $mg/m^3$and 5.0 mg/m^3 groups when compared to control animals. Erythrocyte cholinesterase activity was significantly depressed in both sexes at 5.0 mg/m^3 and in females only at 0.5 mg/m^3 of dichlorvos exposure.

Survival data are summarized in Table 1. Male control rats had a lower survival rate (22 percent) than treated males (30-64 percent). This was also the case for female control rats (47% survival) when compared to treated females (55-72 percent survival). There was a significant increase in survival for both males and females in the 5.0 mg/m^3 group over controls. Many of the animals that died during the course of the study evidenced advanced autolytic changes and were not suitable for necropsy or histological examinations.

The authors reported that a variety of lesions, typical of those commonly found in aged CFE rats, was noted in all groups. A slightly higher tumor incidence was found in animals that were subjected to complete necropsy. Tumors found in all groups included parafollicular cell adenomas and carcinomas of the thyroid gland, anterior pituitary gland adenomas, adrenal pheochromocytomas, and mammary fibroadenomas (females).

TABLE 1: Rat Inhalation Carcinogenesis Study Survival Data
(Blair et al., 1976)

Dichlorvos Chamber Concentration (mg/m^3)	MALES 0	0.05	0.5	5.0	FEMALES 0	0.05	0.5	5.0
Total Rats Examined	50	50	50	50	47[a/]	47[a/]	47[a/]	46[a/b/]
Rats Sacrificed or Dying During Study	39	29	35	18	25	20	21	12
Percentage of Total	78	58	70	36	53	43	45	26
Rats Sacrificed or Dying During Study that were Described as Autolysed	23	13	21	10	6	6	9	4
Percentage of Total	46	26	42	20	13	13	19	9
Rats Sacrificed or Dying During Study that Didn't Receive Complete Postmortem Examination[c/]	23	16	22	8	7	5	10	2
Percentage of Total	46	32	44	16	15	11	21	4
Rats Surviving to Terminal Sacrifice	11	21	15	32	22	27	26	34
Percentage of Total	22	42	30	64	47	57	55	72

a/three animals were used for brain acetylcholine and choline estimations

b/one animal was found to be a male and discarded from the trial (week of termination not given)

c/the meaning of "complete" is not clear. In the narrative of the paper the authors indicate they are referring to those rats "where a complete necropsy and detailed histological examination" was not possible.

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In evaluating the reported negative oncogenic results, several major deficiencies of the study were discovered by the Agency. These include the large number of animals lost to autolysis, the low survival rate in male and female controls (22 percent and 47 percent respectively) compared to the unexplained high survival rate in male and female rats exposed to 5.0 mg/m^3 of dichlorvos (64 percent and 72 percent, respectively), and the less than complete necropsy and histological examination performed on many of the rats. In the male rats, 16-46 percent of the animals in each group did not receive a complete post-mortem examination while, in the females, 4-21 percent received incomplete examination. For those rats given complete examinations, the number and types of tissues taken were not adequately described. It is clear from the data that every organ in each animal was not examined microscopically.

Because of these deficiencies, the lack of oncogenic effects from dichlorvos exposure reported in this study cannot be adequately confirmed. However, Agency pathologists reported that the only neoplasm with an increased incidence in treated females (although not dose-responsive among survivors to autopsy), but not males, relative to controls, was a chromophobe tumor of the pituitary. Initially this was calculated as a statistically significant difference with a p value of 0.052 (Carcinogen Assessment Group 1978). (The level of significance is customarily set at p is equal to or less than 0.05). Further Agency statistical analyses took into account the differential mortality (greater in controls), and indicated significant differences between controls and both the 0.5 mg/m^3 group ($p = 0.043$) and the 5.0 mg/m^3 group($p = 0.039$) in sections examined from all animals. Among survivors to sacrifice, however, no significant differences were found between any treated group and controls. In addition to the statistical uncertainty (occasioned by the many deficiences noted above), and the fact that such hypophyseal tumors are common in aged female CFE rats, no specific biological significance can be attributed to this finding. (Mauer, 1982)

b. Witherup et al., 1967

In the second study reviewed, possible oncogenic effects from chronic oral administration of dichlorvos to rats were examined. Witherup et al. (1967) fed Vapona® insecticide (93 percent dichlorvos, 7 percent unspecified related compounds) at nominal dietary concentrations of 0.1, 10, 100, and 500 ppm to

CD strain rats (7-8 weeks old). Because of the continuous loss of the test compound from the feed (and subsequent slow accumulation of dichloroacetaldehyde), the average weekly concentration of dichlorvos in the diet was approximately 47 percent of the nominal concentration. For the nominal concentrations of 0.1, 1.0, 10, 100, and 500 ppm, the average weekly values of dichlorvos (in ppm) were 0.047, 0.46, 4.67, 46.7, and 234; and of dichloroacetaldehyde (in ppm), 0.014, 0.114, 0.887, 6.86, and 28.6 respectively.

Each dietary group consisted of 40 males and 40 females. Five rats/sex/group were sacrificed at 26, 52, and 78 weeks. The remaining 25/sex/group remained on test for 104 weeks.

Gross and microscopic examinations of tissues were done at sacrifice. Tissues taken included the brain, heart, lungs, liver, spleen, kidney, mammary gland, thyroid, adrenals, gonads, parathyroid, intestine, and pituitary. It was not possible to determine whether these tissues were taken from all animals sacrificed. The type of examinations (if any) performed on animals dying or sacrificed (because of morbidity) during the study was not described. Plasma and erythorocyte cholinesterase activity was determined "periodically" on ten rats from each group.

Although the oncogenic effects of dichlorvos in this study were reported as negative, several features of the experimental design combined to limit the level of sensitivity of this test. The number of animals tested for a full 24 months was inadequate. The rats were seven to eight weeks of age when tested or when testing began. It appears that the maximum tolerated dose (MTD) was not reached in this study. Exposure to dichlorvos at the highest dietary level failed to produce significant weight depression in the high exposure group. The report of the histopathology examination was not presented in detail and was, therefore, inadequate.

c. Witherup et al., 1971

In a separate study by the same authors, five male and five female rats (unspecified strain) were fed a diet containing a concentration of 1,000 ppm of

Vapona® insecticide for two years. (No information was provided on dichlorvos composition.) An equal number of concurrent controls were used (Witherup, et al., 1971).

Plasma and erythrocyte cholinesterase activity was determined at weeks 15 and 104. The animals were sacrificed after two years, and gross and microscopic examinations were performed.

In the test group, four of five females died during weeks 84, 92, and 104. All the males survived for 104 weeks. Overall mortality in the test group was 40% and, in the controls, 44 percent. Growth rate in the females was comparable to that of controls, while the males showed a depressed growth rate. Cholinesterase activity in plasma and erythrocytes was depressed at both 15 and 104 weeks.

These animals suffered from various disease states (chronic interstitial nephritis, chronic bronchitis) unrelated to tumors which were said to occur at approximately the same degree in both control and treated rats. Cytoplasmic vacuolation of the hepatic cells was seen in all test rats (six) examined microscopically. The significance of these alterations was discounted, and they were reported to be variations within physiological limits.

The Agency concluded that because of the poor design and incomplete reporting of study results, only limited information on the toxicity of dichlorvos can be gained from this study. Major deficiencies included the use of only ten animals per dietary level (compounded by low survival in the female test group), testing at only one dietary level, failure to measure actual concentrations of dichlorvos in the animal feed, and the limited reporting of the pathological examination.

d. National Cancer Institute, 1977

The National Cancer Institute, as part of its bioassay program, has tested the oncogenic potential of technical grade dichlorvos (94 percent pure) in Osborne-Mendel rats and B6C3F1 hybrid mice. Stability studies indicated that, kept at 4°C, dichlorvos concentrations after seven days were within 10 percent of theinitial values. Consequently, dietary mixtures were stored at 0°C (or lower)

Table 2: Design of Dichlorvos Chronic Feeding Studies in Osborne-Mendel Rats (NCI, 1977)

Sex and Treatment Group	Initial No. of Animals	Dichlorvos in Diet[a] (ppm)	Time of Study: Treated[b] (weeks)	Time of Study: Untreated[c] (weeks)	Time-Weighted Average Dose[d] (ppm)
Male					
Matched-Control	5	0	0	110	
Low-Dose	50	150	80		150
		0	0	30	
Matched-Control	5	0	0	110	
High-Dose	50	1,000	3		326
		300	77		
		0	0	30	
Female					
Matched-Control	5	0	0	110	
Low-Dose	50	150	80		150
		0	0	30	
Matched-Control	5	0	0	110	
High-Dose	50	1,000	3	30	326
		300	77		
		0	0	30-31	

[a] Doses were lowered because of toxic response.

[b] Treatment periods at high and lowered dosages.

[c] When diets containing dichlorvos were discontinued, treated animals and their matched controls were fed control diets (2% corn oil added) until termination.

[d] Time-weighted average dose = (dose in ppm x no. of days at that dose / no. of days receiving each dose)

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and feed was changed each day. During the study, dietary samples were selected at intervals (24-48 samples/dietary level) and analyzed for dichlorvos concentration. The maximum tolerated dose (MTD) was determined in subchronic feeding studies.

Pooled controls consisted of 60 male and 60 female rats, and 100 male and 80 female mice used as matched controls in concurrent bioassays of aldrin, dieldrin, chlordane, dichlorvos, dimethoate, and heptachlor.

Necropsies were performed on moribund, terminal sacrifice, and dead animals (where possible). The brain, pituitary, adrenals, thyroid, parathyroid, trachea, esophagus, thymus, salivary gland, lymph nodes, heart, lung, spleen, liver, kidney, stomach, pancreas, small and large intestine, urinary bladder, prostate or uterus, testis or ovary, mammary gland, skin, bone (including marrow), and gross lesions were examined microscopically.

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Fisher's exact test and the Cochran-Armitage test for linear trend in proportions, with continuity correction, were used to analyze tumor incidence. Statistical differences in survival between groups were determined by the methods of Cox (1972) and Tarone (1975). The exact 95 percent confidence interval for the ratio between each treatment group and its control was determined by the method of Gart (1970).

1) Rat Bioassay

The experimental design of the chronic feeding study in male and female rats is summarized in Table 2. Since the supplier was unable to provide a sufficient number of rats to start the entire test at one time, one shipment of animals (the high-dietary level group and five matched controls of each sex) was tested beginning at 43 days of age. Four weeks later, a second shipment of animals (the low-dietary level group and five additional control animals of each sex) was obtained from a different supplier (Charles River) than the rats comprising the pooled controls (Battelle). All rats however, were examined histopathologically by the same pathologist. According to the report, "there was probably no significant genetic drift influencing the incidence of tumors" in rats.

During the three week period that the high-level rats received 1,000 ppm dichlorvos, tremors, rough coat, diarrhea, and poor general appearance were observed. Mean body weights for the high-level rats were slightly lower than those for control or low-level rats over the course of the study. At the end of the study, survivors in both the control and treated groups "generally exhibited a poor physical condition." Survival was not dose-related.

A variety of benign and malignant neoplasms commonly seen in aged rats occurred in treated and control animals, however, these lesions were judged to be within the expected range of variation normally seen in aged Osborne-Mendel rats. None of these lesions was considered to be compound related. Statistical analysis of the data (Fisher Test) revealed no statistically significant increase in the tumor incidence in treated animals.

The only significant finding ($p = 0.018$) was a departure from the Armitage and Cochran test for linear trend in proportions in males but not in females with malignant fibrous histiocytomas compared with pooled controls. The incidence of these neoplasms in male rats was 2/58 in pooled controls, 1/10 in matched controls, 4/48 in low-level animals, and 8/50 in high-level animals. Under the conditions of this study, dichlorvos was not found to be carcinogenic in male and female Osborne-Mendel rats. In the judgment of the examining pathologist, "the nature, incidence, and severity of the lesions observed provide no evidence of a carcinogenic effect."

2) Mouse Bioassay

The experimental design of the chronic feeding study in male and female B6C3F1 mice is summarized in Table 3.

During the first two weeks of the study, the mice at 1,000 and 2,000 ppm of dichlorvos showed severe signs of toxicity. After the levels were reduced to 300 and 600 ppm, the appearance and behavior of treated mice were comparable to controls.

A variety of benign and malignant lesions commonly seen in aged mice was observed in all groups. Statistical analysis of the data revealed no significant increase in the tumor incidence of treated mice. Several

Table 1: Design of Dichlorvos Chronic Feeding Studies in NCIFI Mice (NCI, 1977)

Sex and Treatment Group	Initial No. of Animals	Dichlorvos in Diet[a] (ppm)	Time on Study Treated[b] (weeks)	Untreated[c] (weeks)	Time-Weighted Average Dose[d] (ppm)
Male					
Matched-Control	10	0	0	92	
Low-Dose	50	1,000	2		118
		300	78		
		0	0	13	
High-Dose	50	2,000	2		635
		600	78		
		0	0	14	
Female					
Matched-Control	10	0	0	92	
Low-Dose	50	1,000	2		118
		900	78		
		0	0	12-13	
High-Dose	50	2,000	2		635
		600	78		
		0	0	13-14	

[a] Doses were lowered because of toxic response.

[b] Treatment periods at high and lowered dosages.

[c] When diets containing dichlorvos were discontinued, treated animals and their matched controls were fed control diets (2% corn oil added) until termination.

[d] Time-weighted average dose = $\frac{\text{(dose in ppm x no. of days at that dose)}}{\text{no. of days receiving each dose}}$

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proliferative lesions "rarely observed spontaneously in laboratory mice" were reported. These included gastric squamous epithelial hyperplasia in three low-level males and one high-level female, and esophageal papilloma in one high-level female. The occurrence of these lesions, which are rarely seen in aged laboratory mice, prompted additional investigation of sections of esophagus. The incidences of the lesions of the esophagus observed in treated animals, can be found in Table 4. Information on the spontaneous incidence of these lesions was insufficient to establish a relationship between dichlorvos treatment and their incidence.

The evaluating pathologist noted that "an esophageal carcinoma was recently diagnosed in a control mouse of the same strain in another NCI bioassay study". However, adequate historical control data for these tumors in B6C3F1 mice were not available because the esophagus was not routinely examined at that time. Despite the fact that the incidences of esophageal lesions were not statistically significant, these lesions were considered rare in B6C3F1 mice. Under the conditions of this study however, there was insufficient evidence to determine whether the occurrence of lesions of the esophagus in mice was related to the administration of dichlorvos.

3) NCI' Decision to Repeat Tests

The occurrence of consistent mean body weight depression in female and male rats dosed at the high-level and in mice dosed at the high level compared to controls, and slight mean body weight depression in female mice dosed at the high level compared to controls, suggests that the high levels administered to those animals approximated the MTD. However, there were several deficiencies in the experimental design and in the conduct of the studies. Dichlorvos was administered at only two dietary levels and was not administered for the lifespan of the animals. The number of matched controls (10 of each sex) was small compared to the number of animals in each group (50). NCI is currently retesting dichlorvos in Fischer 344 rats and B6C3F1 mice. Substantive results from this study will not be available until February 1983 (Chase, 1982).

Table 4: Esophageal Lesion Incidence in the NCI Dichloroen Bioassay in Mice (NCI, 1977)

	Males		Females	
	Low Level	High Level	Low Level	High Level
Squamous-cell carcinoma	1/47	0/46	0/45	1/41
Papilloma	0/47	0/46	0/45	1/41
Epithelial hyperplasia	1/47	0/46	0/45	0/41

No esophageal lesions were observed in pooled or matched control animals of either sex.

3. Conclusions

No valid scientific study has demonstrated that dichlorvos is carcinogenic. Although negative results were reported in three of the four chronic studies considered for evaluating the oncogenic potential of dichlorvos, all were flawed by major deficiencies in experimental design and inadequate reporting, rendering both data and conclusions insufficient. In the fourth study, equivocal results were reported which have necessitated retesting in the same laboratory (NCI Bioassay). Therefore, the Agency has concluded that existing oncogenic data do not show that dichlorvos has exceeded the risk criteria established for oncogencity.

The Agency has determined that no additional carcinogenicity testing will be required prior to the Agency review of the new NCI bioassay.

B. MUTAGENICITY

1. Introduction

40 CFR 162.11 (a)(3)(ii)(A) provides that " a rebuttable presumption shall arise if the pesticide's ingredient(s), metabolite(s), or degradation product(s) induce "mutagenic effects,as determined by multitest evidence..." The Agency reviewed over twenty papers discussing dichlorvos and mutagenicity.

2. Data Review

There is extensive evidence that dichlorvos is mutagenic in bacteria in the absence of the mammalian metabolic activation system (Table 5). There is some indication that the presence of a metabolic activation system reduces the mutagenicity of dichlorvos in Salmonella typhimurium but not Escherichia coli (Moriya et al., 1978); however, the effects of metabolic activation systems have not been adequately assessed. There is suggestive evidence that dichlorvos is mutagenic in fungi.

In insects, testing is complicated by dichlorvos' powerful insecticidal properties, which reduce the dose levels which can be tested. However, there is some weak evidence that dichlorvos may be mutagenic in Drosophila under

conditions of multigeneration exposure (Hanna and Dyer, 1975), although no mutagenic effect can be detected using customary testing procedures (Kramersand Knaap, 1978; Sobels and Todd, 1979).

Although dichlorvos is mutagenic in microorganisms and may be weakly mutagenic in insects, no mutagenic effects of dichlorvos have been detected in mammalian systems. This finding may be the result of lower sensitivity of the mammalian systems, or metabolic inactivation of the dichlorvos, or both.

Studies representative of the studies described in Table 5 are discussed in this section.

a. Bacterial Studies

1. Gene Mutations

Shirasu et al. (1972; 1976), tested dichlorvos for mutagenicity in Escherichia coli strains WP2 hcr^- and WP2 hcr^+, and in Salmonella typhimurium strains TA 1535, TA 1536, TA 1537, and TA 1538, using 0.1 ml of a 5 percent solution of dichlorvos in dimethylsulfoxide per plate. This treatment did not increase the number of revertants per plate in TA 1536, TA 1537, or TA 1538, but it did increase the number of revertants in both E. coli strains and in TA 1535 as shown in Table 6. This pattern of response is indicative of a base-pair-substitution type of mutagen. The strains of S. typhimurium in which dichlorvos was inactive are sensitive only to frameshift mutagens. No metabolic activation system was used in these studies.

Using a suspension-treatment method, Wild (1973) observed a dose-related increase in mutation to streptomycin resistance in E. coli. Wild incubated E. coli B cells in 0.9 percent saline containing dichlorovs at 10, 15, 20, and 25mM for 1, 2, 3, and 4 hours or at 5 mM for 2, 3, 4, 6, and 10 hours at 37°C without metabolic activation. These treatments produced dose-related increases in the frequency of streptomycin-resistant mutants when the treated cells were plated in the presence of streptomycin at 100 ug/ml. Combinations of time and dichlorvos concentration giving doses of 25 mM x hour or more increased significantly the frequency of mutants above the spontaneous

Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Bacterial Studies					
Reversion spot tests	*E. coli* WP2	Positive	Used pieces of "vapona" strips	Ashton-Smith et al., 1972	Invalid
	E. coli WP2	Positive	Test material 50% dichlorvos, 50% unknown ingredient	Nagy et al., 1975	Invalid
	E. coli WP2	Equivocal negative	Reporting incomplete	Dean, 1972	Invalid
	S. marcescens	Positive at 1.25-5 mg/plate		Dean, 1972	Valid
	E. coli WP2 and derivatives	Equivocal positive	Reporting incomplete	Hanna and Dyer, 1975	Invalid
	S. typhimurium (Ames strains)	Equivocal positive	Reporting incomplete	Hanna and Dyer, 1975	Invalid
Suspension-treatment reversion assay	*E. coli* WP2 and derivatives	Equivocal positive	Reporting incomplete	Bridges et al., 1973	Invalid
Plate-incorporation reversion assay	*E. coli* (W88) (Plasmid-containing)	Positive	Effect detected as low as 0.1 ug/ml	Bridges, 1978	Valid
	E. coli WP2	Positive at 0.1 ml of 5% dichlorvos per plate	Activity not affected by preincubation with cysteine or S-9	Moriya et al., 1978	Valid

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Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Plate incorporation reversion assays (continued)	S. typhimurium TA1535	Positive at 0.1 ml of 5% dichlorvos/ plate	Activity lost by preincubation with cysteine or S-9	Moriya et al., 1978	Valid
	E. coli WP2	Positive at 0.1 of 5% dichlorvos/ plate	No metabolic activation	Shirasu et al., 1976	Invalid
	S. typhimurium (Ames strains)	Positive at 0.1 ml of 5% dichlorvos/ plate	No metabolic activation	Shirasu et al., 1972	Invalid
	S. typhimurium TA100	Equivocal positive at 10 umoles/ plates	Reporting incomplete. Dichloroacetaldehyde (possible metabolite) also equivocal positive at 1.5 umoles/plates	Lofroth, 1978	Invalid
Forward mutation	S. cerevisiae	Positive at 5.6 mg/plate	Only 1 dose tested for streptomycin resistance	Carere et al., 1978	Invalid
	E. coli K-12	Positive at above 10^{-3} M	5-Methyltryptophan resistance	Mohn, 1973	Valid
	E. coli B	Positive at 10 mM for 1 hours	Streptomycin resistance	Wild, 1971	Valid
Fluctuation test (reversion)	E. coli WP2	Positive at 5 ug/ml	Extremely sensitive assay system	Green et al., 1976	Valid

Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Fluctuation test	Various sp.	Equivocal positive at 0.1% dichlorvos	Reporting incomplete	Voogt et al., 1972	Invalid
Host mediated assay (reversion)	S. typhimurium	Equivocal negative	Single low dose No positive control	Voogt et al., 1972	Invalid
Relative toxicity assay	E. coli (various repair-deficient strains)	Positive	Dichlorvos more toxic to polA- than to polA+ exrA- than exrA+ or recA- than recA+	Bridges et al., 1973	Valid
	E. coli polA- and polA+	Positive at 6.4×10^{-4} M	Dichlorvos more toxic to polA- than to polA+ cells	Rosenkranz, 1973	Valid
	B. subtilis recA+ and recA-	Positive with 0.002 ml of 10% DMVP	Dichlorvos more toxic to recA- than to recA+ cells	Shirasu et al., 1976	Valid

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Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Yeast/Fungal Studies:					
In vitro gene conversion dichlorvos	S. cerevisiae	Equivocal positive at 19 mM	Reporting incomplete	Fahrig, 1974	Invalid
	S. cerevisiae D4	Positive at 4 and 8 mg/ml		Dean et al., 1972	Valid
Host mediated assay	S. cerevisiae D4	Negative at 50 and 100 mg/kg and 60 and 99 mg/liter air	Assay of low sensitivity	Dean et al., 1972	Invalid
Forward mutation (8-azaguanine res.)	A. nidulans	Equivocal positive at 14 mg/plate	Reporting incomplete	Bignami et al., 1977	Invalid
	A. nidulans	Equivocal positive at 14 mg/plate	Reporting incomplete	Morpurgo et al., 1977	Invalid
Mitotic recombination	A. nidulans	Equivocal positive at 28 mg/plate	Reporting incomplete	Bignami et al., 1977	Invalid
	A. nidulans	Equivocal positive at 28 mg/plate	Reporting incomplete	Morpurgo et al., 1977	Invalid

Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Mitotic nondisjunction	A. nidulans	Equivocal positive at 28 mg/plate in selective test, and 0.8 mg/ml in non selective test	Reporting incomplete	Rhomad et al., 1977	Invalid
	A. nidulans	Equivocal positive at 28 m/plate	Reporting incomplete	Morpurgo et al., 1977	Invalid
Insect Studies					
Sex-linked recessive lethal	D. melanogaster larvae	Negative at up to 4.1×10^{-4} M	Low doses tested because dichlorvos insecticidal	Kramers and Knaap, 1978	Valid
	D. melanogaster adults	Negative at up to 3×10^{-7} M	Low doses tested because dichlorvos insecticidal	Sobels and Tobi, 1979	Valid
Autosomal recessive lethal	D. melanogaster all stages	Equivocal positive	Population exposed to dichlorvos for 10 generations before testing Nonstandard assay	Hanna and Dyer, 1975	Invalid
Plant Studies					
Cytogenetic	Barley	Equivocal positive at 0.25 - 0.75 mM	Chromosome breaks and fragments may only indicate toxicity	Bhan and Kaul, 1975	Invalid
Chlorophyl mutations	Barley	Negative at 0.25 - 0.75 mM		Bhan and Kaul, 1975	Valid

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Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Mammalian Studies : In vitro cytogenetic test	Human lymphocytes	Equivocal at up to 40 ug/ml	Only toxic response (chromosome degeneration) observed	Dean, 1972	Invalid
In vitro sister chromatid exchange	Human lymphocytes and fibroblasts	Inconclusive (2.5-10 ug/ml)	Interpretation impossible because of solvent effect and deficiencies in reporting and methodology	Nicholas et al., 1978	Invalid
In vivo cytogenetic test	CFI mice	Equivocal negative at 5 ug/liter air, subchronic or 64 and 72 ug/liter air, acute	Methodological defects	Dean and Thorpe, 1972	Invalid
	Chinese hamsters	Equivocal negative at 32 ug/liter air, acute; 10 and 15 mg/kg, acute, oral	Methodological defects	Dean and Thorpe, 1972	Invalid

Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Dominant lethal assay	Female CFI mice	Negative at 25 and 50 mg/kg, acute oral; 2 and 8 ug/liter air, 8 weeks	Nonstandard assay Numbers of animals low	Dean and Blair, 1976	Invalid
	Male CFI mice	Negative at 30 and 55 ug/liter air, 16 hours; 2.1 and 5.8 ug/liter air, 4 weeks	Numbers of animals low	Dean and Thorpe, 1972	Invalid
	Male ICR/Ha mice	Equivocal negative 5 and 10 mg/kg/day, 5 days; and 13 and 16.5 mg/kg acute i.p.	Reporting incomplete Low sensitivity Numbers of animals	Epstein et al., 1972	Invalid
Sperm morphology test	Male hybrid mice	Equivocal positive at about 7 and and 13 mg/kg	Reporting incomplete Relevance unclear	Wyrobek and Bruce, 1975	Invalid

Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
Relevant Biochemical Tests					
Chemical reactivity	Nucleophilic chemicals	Positive	Dichlorvos reacts with nucleophiles in vitro ;hence may react with DNA	Jentzch and Fischer, 1974 Lofroth et al., 1969	Valid
NBP reaction	4-(4 nitrobenzyl)-pyridine	Positive	Implies dichlorvos may react with DNA	Fischer and Lohs, 1973	Valid
DNA breakage	plasmid DNA in vitro	Positive at 1 mg/ml	Implies dichlorvos can damage DNA in vitro	Griffin and Hill, 1978	Valid
DNA strand breakage	E. coli cells In vitro	Positive at about 0.2%	Dichlorvos damages E. coli DNA	Green et al., 1974	Valid
	Chinese hamster cells	Positive at 0.2%	Dichlorvos can cause DNA damage in Chinese hamster cells in vitro	Green et al., 1974	Valid
DNA alkylation test	Isolated DNA	Positive at about 7-14 mM	Dichlorvos damages E. coli DNA In ether solution, some O^6-methylguanine formed	Lawley et al., 1974	Valid
	E. coli cells	Positive at 2.9 mM	Dichlorvos alkylates DNA in bacteria, no O^6-methylguanine detected	Lawley et al., 1974	Valid

Table 5: Dichlorvos Mutagenicity and Other Genetic Toxicity Studies (continued)

Assay Type	Organism	Outcome	Comments	Reference	Evaluation
DNA Alkylation Test (Continued)					
	E. coli cells	Positive at 1.1 mM	Dichlorvos alkylates DNA in bacteria forming 7-methylguanine	Wennerberg and Lofroth, 1974	Valid
	Human carcinoma (HeLa) cells	Positive at 0.97 mM	Dichlorvos alkylates DNA in HeLa cells forming 7-methylguanine	Lawley et al., 1974	Valid
	Male rats	Equivocal negative at 0.064 ug/liter air for 12 hours	Single low dose tested; low sensitivity	Wooder et al., 1978	Invalid
Purine alkylation	Male mice	Positive	7-methylguanine in urine but source equivocal, suggests DNA alkylation	Wennerberg and Lofroth, 1974	Invalid
	Male rats	Positive at 0.07 mole/rat	7-methylguanine and 3-methyl-adenine in urine suggest dichlorvos alkylates DNA but not conclusive	Lofroth and Wennerberg, 1974	Invalid

Table 6: Induction of Revertants in Escherichia coli and Salmonella typhimurium by Dichlorvos (Shirasu et al., 1976)

Treatment	Revertants per plate		
	E. coli her+	E. coli her-	S. typhimurium TA 1535
Solvent	15	37	8
0.1 ml, 5% dichlorvos	281	113	305

frequency of 2-5/10^9 cells ($p = <0.01$). For example, treatment with 10 mM of dichlorvos for 1, 2, 3, and 4 hours produced mutation frequencies of 1.8, 4.0, 5.8, and 11.5/10^9 cells, respectively; with 20 mM of dichlorvos, the corresponding frequencies were 2.9, 13.0, 30.3, and 40.9/10^9 cells, respectively. The effects of a mammalian metabolic activation system were not examined in this study.

Using an extremely sensitive fluctuation test system with E. coli WP2, Green et al. (1976), were able to detect an increase in the mutation rate induced by concentrations of dichlorvos as low as 5 ug/ml. No metabolic activation system was used.

In the only bacterial study in which a mammalian metabolic activation system was used, Moriya et al. (1978) incubated a Japanese commercial sample of dichlorvos with water or with a complete rat liver microsome metabolic activation system (S-9) for up to 60 minutes at 37°C before adding S. typhimurium strain TA 1535 or E. coli strain WP2 and plating to measure the induction of revertants by dichlorvos (equivalent to 0.1 ml of a 5 percent solution per plate). As shown in Table 7, incubation with the S-9 mix eliminated the mutagenic effect of dichlorvos in S. typhimurium, but did not affect this activity in E. coli.

Similar effects were observed when dichlorvos at 22.6 u moles/plate was preincubated with 20 mM of cysteine for 60 minutes at 37°C; the mutagenic effect in S. typhimurium was abolished, but the effect in E. coli was not.

2. DNA Damage and Repair

Rosenkranz (1973), treated growing cultures of E. coli that possessed or lacked a functional gene (polA) for DNA polymerase 1 (repair enzyme) with 6.4 mM of dichlorvos and counted the number of viable cells at 0.5, 1, and 2 hours by plating aliquots on nutrient agar. In the absence of dichlorvos both the polA$^+$ (repair-competent) and polA$^-$ (repair-deficient) strains grew. They increased from 1.1 to 9.3 x 10^8/ml and from 1.4 to 8.7 x 10^8/ml, respectively, in 2 hours. When dichlorvos was added, the number of viable cells of the polA$^+$ strain declined about 10-fold in two hours to 1 x 10^7/ml; in the polA$^-$ strain, the decline in viable cells was almost

Table 7: Incubation of S-9 Mix with Salmonella typhimurium and Escherichia coli (Moriya et al., 1978)

Incubation	Revertants per plate in			
	S. typhimurium		E. coli	
Time (min.)	H_2O	S-9 mix	H_2O	S-9 mix
0	212	12	130	130
10	186	11	176	151
30	134	8	142	125
60	138	6	120	147

1,000-fold to 1.5 x 10^5ml. This finding suggests that dichlorvos damages DNA and that this damage is repaired less well in the polA$^-$ than in the polA$^+$ strain. DNA polymerase 1 is involved in the excision repair of DNA damage (Witkin, 1976).

Similar effects were observed by Shirasu et al. (1976) in the rec$^-$ assay with Bacillus subtilis. When 0.02 ml of a 10 percent solution of dichlorvos was placed at the origin of streaks of B. subtilis strains H 17 rec$^+$ and M 45 (rec$^-$), no zone of inhibition was produced in the rec$^+$ strain, but a zone of inhibition 9.5 mm long was produced in the rec$^-$ strain. This indicates that dichlorvos was more toxic to the recombination repair-deficient (rec$^-$) than to the repair-proficient (rec$^+$) strain of B. subtilis, and suggests that dichlorvos causes DNA damage that can be repaired in the rec$^+$but not in the rec$^-$strain. Neither of the above studies used a mammalian metabolic activation system.

b. Fungal Studies

Bignami et al. (1977), performed a series of genetic tests with dichlorvos using the fungus Aspergillus nidulans. The authors reported the results of their experiments rather briefly but indicated that dichlorvos at 14 mg/plategave positive results in a spot test for the induction of mutants resistant to 8-azaguanine; and at 28 mg/plate, gave strongly positive results in a spot test for mitotic crossing over. In a nonselective test for nondisjunction, dichlorvos at 0.8 mg/ml, a dose that did not reduce survival, increased the frequency of nondisjunction from 4/120 among solvent-treated colonies to 35/75 among colonies of cells treated with dichlorvos. Because of the incomplete reporting, these data are not definitive, but they are consistent with the bacterial data, and they confirm that dichlorvos has mutagenic and other genotoxic effects.

Further evidence of the genetic activity of dichlorvos comes from a study by Dean et al. (1972). These authors treated cells of the yeast Saccharomyces cerevisiae D4 with dichlorvos at 0.25, 5, 1, 2, 4, and 8 mg/ml for 5 hours and tested the cells for gene conversion at the ade 2 and trp 5 loci. Treatments with dichlorvos at 4 and 8 mg/ml significantly increased the

frequency of gene conversion above the control level. Positive results were also obtained with the positive control chemical ethyl methanesulfonate (EMS) at dose levels of 1, 2, 4, and 8 mg/ml.

Dean et al. (1972) also performed a host-mediated assay for gene conversion with the same strain of S. cerevisiae in mice. Positive results were obtained with the positive control, EMS, at 400 mg/kg, the only dose tested. Although negative results were obtained with dichlorvos, the highest dose tested was only 100 mg/kg, although this was not explicitly stated. If the comparative activity of dichlorvos and EMS observed in vitro were maintained in vivo, the dose of dichlorvos tested in the host-mediated assay would probably be too low for any statistically significant activity to be detected.

c. Studies in Insects

Because of its powerful insecticidal properties, only low concentrations of dichlorvos can be tested in insects. In Drosophila melanogaster, using standard techniques for detecting sex-linked recessive lethal mutations, no increase in mutation frequency could be detected with the highest testable doses: 4.1×10^{-7} M (0.09 ppm) in larval feeding studies (Kramers and Knaap 1978), and 3×10^{-7} M (0.066 ppm) in adult feeding studies (Sobels and Todd, 1979).

Hanna and Dyer (1975), however, did obtain indications of a mutagenic effect of dichlorvos in D. melanogaster by feeding flies over 30 generations with increasing concentrations of dichlorvos up to 0.75 ppm as some degree of tolerance developed, and then testing at 18 months (0.75 ppm level), for recessive lethal mutations on the second chromosome. The frequency of recessive lethals in the dichlorvos-treated population was 42/130 (32.3 percent), significantly greater ($p = 0.01$) than in the concurrent control population (9.2 percent) compared to established control values of 0.12 to 1.75 %. It is unclear whether the control population received the solvent for dichlorvos or were untreated.

d. Mammalian Studies

The mutagenicity of dichlorvos has been studied in mammalian systems with equivocal results.

Cytogenetic studies have been performed with human lymphocytes *in vitro* (Dean, 1972) and in mice and Chinese hamsters *in vivo* (Dean and Thorpe, 1972). Dominant lethal assays have been performed in mice, treating both males (Dean and Thorpe, 1972; Epstein et al., 1972) and females (Dean and Blair, 1976). No significant mutagenic effect was detected in any of these studies at the highest dose levels tolerated.

e. Relevant Biochemical Tests

Lofroth et al. (1969) first proposed that dichlorvos is an alkylating agent capable of reacting with various nucleophiles, possibly including nucleophilic sites in DNA. Since many alkylating agents are mutagens and some are carcinogens (Miller and Miller, 1971), the finding that dichlorvos is an alkylating agent raised concern that it might present a mutagenic or carcinogenic hazard. More recent studies have confirmed that dichlorvos is a weak alkylating agent (Jentzch and Fischer, 1974), and that it can alkylate isolated DNA, as well as DNA in bacteria and mammalian cells in culture (Lawley et al., 1974). The major site of DNA alkylation was the N^7-position of guanine. 7-Methylguanine was the only product detected in mammalian cells, although low levels of other products, including O^6-methylguanine, a possible premutational lesion, were detected in the isolated DNA and in the bacterial cells treated with dichlorvos (Loveless, 1969).

Studies of DNA alkylation with intact mammals are few in number and have given equivocal results. Wooder et al. (1978) exposed rats to atmospheres containing "practical use concentrations" of ^{14}C-methyl labeled dichlorvos (0.064 ug/liter of air) for 12 hours (total inhaled dose estimated as about 6 ug per rat). After exposure the animals were killed, and their tissues were homogenized. Protein, RNA, and DNA fractions were separated, and their ^{14}C-content was measured. The DNA and RNA fractions were also hydrolyzed and again fractionated. The low level of labeling associated with the hydrolyzed nucleic acids did not appear to be associated with any of the peaks of the DNA bases or with the peak of added 7-methylguanine. This indicates that under the conditions of exposure no specific methylation of the DNA or RNA bases was detected (limit of detection: 1 methylation per 6×10^{11} nucleotides of DNA or per 2×10^9 nucleotides of RNA).

Lofroth and Wennerberg (1974), and Wennerberg and Lofroth (1974), did detect ^{14}C-labeled 7-methylguanine in the urine of treated rats and mice, which were given doses of ^{14}C-labeled dichlorvos 25-500 times greater than that used by Wooder et al. (1978). Although this suggests that dichlorvos alkylated DNA, or RNA, it is not entirely clear whether this 7-methylguanine in the urine actually resulted from the breakdown of DNA or RNA methylated by dichlorvos, from direct alkylation of free guanine, or from the breakdown of dichlorvos and utilization of the liberated ^{14}C label in normal biosynthesis.

3. Conclusions

There is valid multitest evidence that dichlorvos is mutagenic, as well as inducing DNA damage in microorganisms, and may be a direct alkylator of bacteria and fungal DNA and RNA in vitro. However, no mutagenic effects have been detected in mammalian systems, either in vitro or in vivo (Mauer, 1981).

In the absence of a validated test, showing mutagenicity in mammalian systems,the Agency concludes that dichlorvos does not meet the "validated test or othersignificant evidence raising prudent concerns..." criteria specified in Section3(c)(8) of the FIFRA (1978) amendments.

The following tests are necessary to definitively determine the potential mutagenic effects of dichlorvos in mammals:
... In vitro mammalin cell mutation assay with L5178Y TK+/- or CHO HGPRT, or other established cell system.
... In vitro cytogenetics tests, e.g. micronucleus test or bone marrow chromosome aberration assay.
... Rodent dominant lethal assay.
... Sister-chromatid exchange, in vitro or in vivo . In in vitro assays, inclusion of a metabolic activation system will be requested to assess the apparent weak mutagenic effect of dichlorvos.

C. Teratogenicity and Fetotoxicity

1. Introduction

40 CFR 162.11 (a)(3)(ii)(B) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s) "produces any other chronic or delayed effectin test animals at any dosage up to a level, as determined by the Administrator which is higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety."

2. Data Review

Notwithstanding evidence suggesting that dichlorvos may cross the placental barrier to alter fetal OP-sensitive enzyme systems (Maslinska and Zalewska, 1978; Starzye and Kifer, 1974), no dichlorvos treatment-related teratogenic or fetotoxic effects have been demonstrated in studies specifically conducted to assay teratogenicity.

Of the dozen studies reviewed for teratogenic and/or fetotoxic potential of dichlorvos, ten have been determined to be invalid because of basic experimental design flaws (See Table 8). The two valid studies demonstrated no dichlorvos-related teratogenic-fetotoxic effects in the absence of severe maternal toxicity in mice (Schwetz et al., 1979), in rats (Thorpe et al., 1972), and in rabbits (Schwetz et al., 1979; and Thorpe et al.,1972). Avian studies (Roger et al., 1969; Khera and Lyon, 1968; Proctor et al., 1976; Seifert and Casida, 1978) are not considered relevant for assessing teratogenic potential in mammals.

The value of 15 mg/kg given as the TDLo (low toxic dose) for "mice", for example, as cited in the 1979 NIOSH "Registry of Toxic Effects of Chemical substances," actually derives from the experimentally inadequate study of Kimbrough and Gaines (1968), who reported a marginal increase in malformations (3 cases of omphalocele among 41 treated pups ($p = 0.055$)), after intraperitoneal administration of a single 15 mg/kg dose on day 11 to pregnant Sherman rats.

Table 8: Teratogenicity Studies With Dichlorvos

Study	Species	Route	Dose[a]	Reported Result	Evaluation
1. Beyer et al. (1969)	Chicken	Intra-ovo	10 x 1	Positive	Invalid
2. Kimbrough & Gaines (1968)	Rat	Intraperitoneal	15 x 1	Positive	Invalid
3. Thorpe et al. (1972)	Rat	Inhalation	0.25, 1.25, 6.25 x 20	Negative	Valid
	Rabbit	Inhalation	0,2,4 x 28	Negative	Valid
4. Baksi (1978)	Rat (Tx/F1)	Oral	25 x 1	Negative	Invalid
5. Khera & Lyon (1968)	Chick/Duck	Intra-ovo	0.01-1.0 x 4	Negative	Invalid
6. Proctor et al. (1976)	Chicken	Intra-ovo	1 x 1	Negative	Invalid
7. Schwetz et al. (1979)	Mouse	Oral	60 x 10	Negative	Valid
	Rabbit	Oral	5 x 13	Negative	Valid
	Rabbit	Inhalation	4 x 13	Negative	Valid
8. Starzye & Kifer (1973)	Rat	Oral	"50" x 13/20 (formulation)	Negative	Invalid
9. Duneska et al. (1979)	Rabbit	(?)	(?)	Negative	Invalid
10. Maslinska & Zalewski (1978)	Rabbit	(?)	6 x 10	(-)	Invalid
11. Seifert & Casida (1978)	Chicken	Intra-ovo	1 x 1	Negative	Invalid
12. Young et al. (1979)	Swine	Feed	4 x 30	Negative	Invalid

[a] Dose: mg/kg or g/L, times days
(?) = Not Given

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From the studies adjudged valid, the "no observed effect levels"(NOEL) in certain mammalian species are outlined in Table 9 (Mauer, 1982).

3. Conclusion

A comprehensive examination of the data base reveals no definitive evidence or valid tests suggesting that dichlorvos induces teratogenic-fetotoxic effects in the absence of maternal toxicity.

No additional teratology studies will be required at this time.

D. Reproductive Effects

1. Introduction

40 CFR 162.11 (a)(3)(ii)(B) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s) "produces any other chronic or delayed effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety."

2. Data Review

The available data base contained only two long-term reproduction studies, both employing the dietary route of administration (Singh et al., 1966, in Yorkshire and Landrace/Hampshire swine; and Witherup et al., 1965, in Charles River rats). In neither study were adverse effects found in reproductive performance or other parameters throughout two or three generations of feeding dichlorvos at levels up to 500 ppm.

Intragastric administration of comparable daily dosages (ca. 25 mg/kg dichlorvos) for shorter periods of time (ca 30 days) were also without reproductive consequences in pigs (Stanton et al.,1979; Young et al., 1979).

Table 9: No Observed Effect Level (NOEL) For Fetotoxicity/ Teratogenicity With Dichlorvos (Mauer, 1982)

Route	Species: Rat	Mouse	Rabbit	Reference
Inhalation (mg/l)	6.25		4.0	Thorpe et al. (1972)
Oral (mg/kg/day)	- -	60.0	5.0	Schwetz et al. (1979)

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Evidence from a number of short-term studies suggests the induction of transient reproductive effects, but the purported positive results are considered equivocal because of major deficiencies in experimental design and inadequate reporting.

Timmons et al. (1975) exposed CD rats to dichlorvos in the form of "No-Pest Strips" from birth, and reported a significant increase averaging 10 days in the appearance of first estrus, as manifested by presence of sperm and/or cornified epithelial cells (52 $\pm$ 2.3 days vs 42 $\pm$ 2.2 days in controls). However, the unknown (and unreported) concentration of dichlorvos volatilized from the strips, combined with the known variability of first estrus in rodents among other methodological deficiencies render the validity of this delay questionable.

Apparent interference with spermatogenesis and/or frank damage to testicular tissues in rats and mice has been reported at dosage schedules comparable to those used in chronic and subchronic reproduction studies which have demonstrated no dichlorvos-related spermatogenic changes, either orally or by inhalation. In a series of studies by Krause and associates (Krause and Homola, 1972, 1974; Krause et al., 1976; Krause, 1977), "seminiferous tubule damage" and other "spermatogenic abnormalities" were reported immediately following oral administration (by gavage) of dichlorvos, but were reported no longer histologically detectable three to four weeks in post-treatment". Thus, the transient nature of these disturbances, as well as other inadequacies of protocol details, render the reported positive results equivocal.

Wyrobeck and Bruce (1975) reported abnormally shaped sperm heads in NMRI mice treated intraperitoneally for 5 days at doses of 1, 3, 7, and 13 mg/kg. Although the study appears to have been performed in an appropriate manner, the relation between dose and resulting frequencies of abnormality is unclear. Equally equivocal is the reproductive significance of this finding (applicable also to the Krause studies cited above), since reproductive performance of treated mice was not tested. No other clinical effects were reported in any of these short-term studies.

In a well-controlled dermal study using albino rats with clipped hair by Dickshith et al. (1976), no spermatogenic disorders or lesions were found. They were serially painted for 65 days with a dichlorvos preparation providing 21.4 mg/kg exposure.

Although none of the tests for dominant lethality (Dean and Blair, 1976; Dean and Thorpe, 1972; Epstein et al., 1972) can be considered adequate to support the negative results reported, there is also no suggestion of a spermatogenic effect attributable to dichlorvos.

The remainder of the studies reviewed from the available literature are of dubious validity, and indicate no significant reproductive effects in treated animals (Table 10). The NOEL's for reproductive effects based on the results of Witherup et al., 1965, and Dickshith et al., 1976, can be estimated as:

(i) From chronic dietary studies (rat) .....(greater than) 500 ppm.
(ii) From subchronic dermal studies (rat).....(greater than) 21.4 mg/kg (Mauer, 1982)

3. Conclusions

A comprehensive examination of the reproductive data base reveals no definitive evidence or valid tests suggesting dichlorvos has any significant adverse effect on fertility or other reproductive parameters. Therefore, no additional assays for reproductive effects are required at this time.

E. Neurotoxicity

1. Introduction

40 CFR 162.11 (a)(3)(ii)(B) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s) "produces any other chronic or delayed effect in test animals at any dosage up to a level, as determined by the Administrator,which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety."

Table 10: Reproduction Studies With Dichlorvos

Study	Species	Route	Dose*	Reported Result	Evaluation
1. Singh et al. (1966)	M/F Swine	Feed	250-500	Negative	Invalid
2. Witherup et al. (1965) (Same rat data as Witherup et al., 1972)	M/F Rat 1 generation/2 years	Feed	0.1-500 ppm/day	Negative	Valid
3. Stanton et al. (1979)	M/F Swine	Oral	25 x 80 (TC) Also: 1,4, 16 x 80 (PVC-FP)	Negative	Invalid
4. Young et al.(1979)	Swine	Feed	4 x 30	Negative	Invalid
5. Timmons et al. (1975)	Rat	Inhalation?	(?) x 40 +	Negative	Invalid
6. Krause & Ikenola (1972/74)	M mouse	Oral	40 x 1; also 10 x 18	Positive	Invalid
7. Krause (1977)	M rat	Oral	10 x 6; also 5 x 9	Positive	Invalid
8. Wyrobek & Bruce (1975)	M mice	Intraperitoneal	1, 3, 7, 13 x 5	Positive	Invalid
9. Krause et al. (1976)	M Rats	(Oral?)	20 x 2; also 10 x 20	Positive	Invalid
10. Dickshith et al. (1976)	M Rats	Dermal	21.4 x 65	Negative	Valid
11. D'Souza & Batra (1976)	Mice	Inhalation/dermal (Cages sprayed)	(?)	Positive	Invalid
12. Guthrie et al. (1971)	Mice	Intraperitoneal	(?)	Negative $(LD)_{50}$ = 40-50	Invalid
13. Dean & Blair (1976)	F Mice	Oral Inhalation	25, 50 x 1 2, 8 x 56	Negative Negative	Invalid Invalid
14. Dean & Thorpe (1972)	M Mice	Inhalation Inhalation	80, 55 x 1 2.1, 5.8 x 20	Negative Negative	Invalid Invalid
15. Epstein et al. (1972)	M Mice	Oral Intraperitoneal	5, 10 x 5 11, 16 x 1	Negative (? Negative)	Invalid Invalid

(?) Information Not Given.
* Dose, ug/l. for inhalation, mg/kg for other routes, x number of days treated.

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2. Data Review

The following three studies were performed with dichlorvos in chickens and are uniformly negative for delayed neurotoxicity.

Aldridge and Barnes (1966) tested 22 organophosphate compounds in adult hens for delayed neurotoxicity. The hens were pretreated with atropine (25 mg S.C.) and P2S (100 mg IP) before dosing to protect them from the acute toxic effects of the test compound. A single 25 mg dose of dichlorvos S.C. produced no signs of delayed neurotoxicity during a 21-day observation period.

Witherup et al., (1963) reported the toxic effects of dichlorvos (Vapona) in White Leghorn hens. The single dose oral LD_{50} was determined to be 22.8 $\pm$1.6 mg/kg. Hens treated with a single oral dose either died or recovered in 24 hours. No late developing abnormalities indicative of delayed neurotoxicity were observed in these hens. Fourteen hens were given oral doses of dichlorvos at 2.5 mg/kg five days per week for three weeks. No signs of delayed neurotoxicity were observed during dosing and a subsequent observation period.

Durham et al., (1957) reported the toxicity of dichlorvos in several species. Rhode Island Red hens were pretreated orally with atropine and then dosed with dichlorvos subcutaneously. Eight hens dosed with 15 mg/kg showed no signs of delayed neurotoxicity during a subsequent observation period.

These studies represent all the studies of dichlorvos in chickens that are available and are sufficient to demonstrate a lack of delayed neurotoxic effect.

All available toxicity studies of dichlorvos in mammalian species which were purporting to show a neurotoxic effect have been examined (Aldridge and Barnes, 1966; Brzezinski, 1972; Fatehyab et al., 1979; Hazelwood et al., 1979; Johnson, 1970; Maslinska and Zalewska, 1978; Weintraub et al., 1976). The only effect shown in these studies is secondary to cholinesterase inhibition and, if not lethal, is reversible when dichlorvos administration is stopped.

3. Conclusion

The information available is sufficient to show that dichlorvos, by itself, does not produce organophosphate type delayed neurotoxicity. No additional studies on the neurotoxicity of dichlorvos alone are required; however the Agency reserves the right to request at some later date such studies involving dichlorvos and other pesticides where there is evidence of potentiation and/or there is considerable likelihood of concurrent exposure.

IV. AGENCY CONCLUSIONS

A. Conclusion Concerning Dichlorvos And The RPAR Risk Criteria

The Agency has reviewed four studies of the oncogenic potential of dichlorvos. No positive evidence of oncogenicity was identified. The studies were, however, all flawed requiring additional information. The National Cancer Institute is conducting an evaluation of the oncogenic potential of dichlorvos. No additional data will be required of the registrants until the Agency has reviewed the results of the NCI study.

The Agency has reviewed the mutagenicity data on dichlorvos. There is extensive evidence that dichlorvos is mutagenic in bacteria in the absence of a mammalian activation system. There is also suggestive evidence that dichlorvos is mutagenic in fungi. However, no mutagenic effects of dichlorvos have been detected in mammalian systems. Additional testing will be required to confirm the lack of mutagenic effects in mammalian systems.

An evaluation of the available data reveals no definitive evidence or valid tests suggesting that dichlorvos induces teratogenic or fetotoxic effects in the absence of maternal toxicity.

An evaluation of the reproductive effects data reveals no definitive evidence or valid tests suggesting dichlorvos has any adverse effect on fertility or other reproductive parameters.

The available information is sufficent to show that dichlorvos does not produce organophosphate type delayed neurotoxicity.

Based on the above information, the Agency has determined that the existing evidence does not support the issuance of an RPAR for dichlorvos and consequently, that RPAR's for trichlorfon and naled as precursors of dichlorvos are also not warranted. No direct evaluation of trichlorfon or naled was conducted.

B. Additional Toxicology Data To Be Required

Equivocal data are present with respect to two toxic effects. The two effects are carcinogenicity and mutagenicity. The Agency will wait until the NCI bioassay on carcinogenicity is completed and reviewed prior to determining if additional data on carcinogenicity are to be required of the registrants.

Concerning the potential mutagenic effects, the Agency will seek confirmation that dichlorvos does not transport to the germ cells. The following tests are required to provide data on this question:

1. In vitro mammalian cell mutation assay with L5178Y TK+/- or CHO HGPRT, or other established cell system,
2. In vivo cytogenetics test, e.g. micronucleus test or bone marrow chromosome aberration assay in mice or rats,
3. Rodent dominant lethal assay,
4. Sister-chromatid exchange, in vitro or in vivo ,

With in vitro studies, inclusion of a metabolic activation system will be requested to assess the apparent weak mutagenic effect of dichlorvos.

These tests will be required of the registrants under the provisions of FIFRA 3(c)(2)(B).

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